Applicant thanks the Examiner for the withdrawal of the rejections under 35 U.S.C. § 102(b).

Rejection under 102(e)

Claims 1, 2, 8-13, 16, and 21-22 stand rejected under 35 U.S.C. § 102(e) as being allegedly anticipated by Portnoy et al. (US Patent No. 6,004,815). The Examiner states at p. 3 of the Office Action:

> Portnoy et al. disclose an attenuated derivative of a pathogenic mircroorganism (*E. coli*) (see abstract, table 1 and claims 1-6), plasmid vectors (column 8, table 2) and gene operably linked to an eukaryotic promoter (CMV)(see column 3). They teach *E. coli* deficient in the production of DAP (see column 16) and a recombinant complementing gene on a vector (plasmid pWR100 from *Shigella flexneri*)(column 16).

Applicant respectfully maintains that the Portnoy reference does not teach a vector with a recombinant complementary gene. The bacteria shown in Table 1 and described in other parts of Portnoy are merely attenuated and do not possess a non-functional native chromosomal essential gene, as the attenuated bacteria remain viable. The Examiner points specifically to column 16, where *E. coli* deficient in the production of DAP are mentioned. The Examiner alleges that the pWR100 plasmid provides the complementary gene. However, in Portnoy, at column 16, lines 45-52, the DAP-minus *E. coli* that contain plasmid pWR100 "...have the ability to invade cultured cells and enter the cytosol..., yet following brief replication, spontaneously lyse in the cytosol..." Accordingly, the pWR100 merely allows the *E. coli* to invade the cell and enter the cytosol. The *E.coli* lyse even in the presence of pWR100. A cell containing a plasmid that carries a recombinant complementary gene would produce DAP such that the cell would not spontaneously lyse. The plasmid pWR100, therefore, does not carry a recombinant complementary gene. In fact, for pWR100, Portnoy refer to Courvalin et al., C.R. ACAD. SCI. PARIS 318:1207-12 (1995), which refer to Sansonetti et al., INFECT. IMMUN. 15:852-60 (1982). The pWR100, as taught in Sansonetti, does not carry a recombinant complementary essential gene. See also Buchrieser et al., *The virulence*

plasmid pWR100 and the repertoire of proteins secreted by the type III secretion apparatus of Shigella flexneri, MOL. MICROBIO. 38(4):760-771 (2000). (submitted in an IDS enclosed herewith).

Finally, the plasmids in Table 2 in Portnoy et al. do not carry a complementary essential gene. Instead, pDP3615 carries a gene that encodes listeriolysin O (LLO). (col. 9, lines 14-16). LLO provides a vehicle to deliver co-encapsulated protein to the cytosol of macrophages. (col. 7, lines 39-44). The vector pDP3616 carries a gene that encodes for chicken ovalbumin (OVA). (col. 9, lines 33-36). OVA is used as a mock foreign gene to demonstrate the delivery of protein to the cytosol of macrophages. (col. 8, lines 34-38). The vector pDH70 carries a promoterless lacZ gene. LacZ is a well-known marker. The present specification defines an "essential" gene as one that encodes a function that is required for cell viability. LLO, OVA, and LacZ are not essential genes because they are not required for cell viability. The other plasmids in Table 2 are just cloning vectors with antibiotic resistant genes. Accordingly, Table 2 does not teach any vector with a recombinant complementary gene. Thus, the Portnoy et al. reference fails to teach each element of the claimed invention, and therefore, does not anticipate the instant claims.

Claims 1-7, 12-20 and 45-46 stand rejected under 35 U.S.C. § 102(e) as being allegedly anticipated by Curtiss III et al. (U.S. Patent No. 6,024,961). Specifically, the Examiner states at p. 4 of the Office Action:

> Curtiss III et al. (US Patent No. 6024961) disclose an avirulent immunogenic strain of *Salmonella enterica* serotype *Typhi* having a mutation in one or more genes comprising of pab, pur, aro, asd, dap, nadA,.... They also teach a recombinant gene encoding the desired gene product (see column 11). They disclose bacterial antigens (column 11). They also disclose recombinant vectors (example 2, column 28) and desired gene product cytokine (columns 10 and 11).

The currently pending claims, e.g. claim 1, recite "wherein the complementing gene can recombine to replace the non-functional chromosomal essential gene..." Column 11 of the cited '961 patent summarizes the balanced-lethal system described in U.S. Patent No. 5,672,345. Example 6 of the '345 patent describes a means of generating

Δ*asd* mutations by excision of a Tn*10* linked closely to the *asd* gene. As described in the '345 patent, the mutation is due to Tn*10* insertion into a DNA sequence adjacent to the *asd* gene, and then the Δ*asdA1* mutation arose by fusaric acid resistance selected deletion of the Tn*10* and adjacent gene sequences that included the *asd* gene. As a result, DNA sequences flanking the *asd* gene were deleted also, making it impossible for a wild-type *asd* gene, such as is carried on plasmid pYA3148 or pYA3193, to recombine to functionally replace the deleted *asd* mutation. (It was because of this description that the Examiner, during prosecution of the '345 patent, required that the claims of that patent stipulate the inability of the vector *asd* gene to recombine to replace the *asd* chromosomal mutation).

Moreover, the '345 patent teaches away from the present invention. The patent teaches that "integration of the recombinant gene into the chromosome overcomes many of the potential benefits of having it reside on the plasmid." (col. 3, lines 6-26). Based on this teaching, a person of ordinary skill in the art would not be motivated to produce a balanced lethal host-vector system of the present invention, in which the extrachromosomal vector or plasmid can recombine with the chromosome.

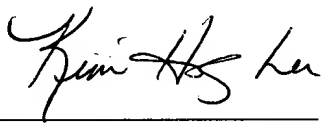
As described in the Examples of the instant application, newer techniques of generating defined deletion mutations by allele replacement enable deletions of just the structural *asd* gene. Thus, when Asd+ vectors with the entire *asd* gene and its flanking 5' promoter and 3' termination sequences are used to complement the *asd* chromosomal mutation it is at least possible that the plasmid-born *asd* gene can recombine to replace the chromosomal mutation, compromising the functional utility of the system. The studies described in the instant application demonstrate that such a system does in fact retain utility, because although recombinational replacement of the chromosomal *asd* mutation is possible, it occurs at such a low frequency as to not compromise the utility of the claimed functional balanced-lethal host-vector system. Thus, this feature (retention of functional utility despite the ability to recombine) constitutes a novel and non-obvious difference between the claimed composition and the composition of the prior art. Example 2 of the '961 patent, as cited in the Examiner's rejection, does not teach this concept. The example does not teach that an extrachromosomal vector carrying a

complementary copy of the essential gene can recombine with the chromosome. The example merely teaches how to create a deletion mutation.

CONCLUSION

Applicant requests entry of the foregoing amendments and remarks into the file of the above-identified application and respectfully requests withdrawal of the Examiner's rejections. Applicant believes he has overcome or obviated all of the Examiner's rejections. Applicant submits that the application is in proper condition for allowance, and respectfully requests that such allowance be granted.

Respectfully submitted,



Kimberly H. Lu
Reg. No. 51,973
Thompson Coburn LLP
One US Bank Plaza
St. Louis, MO 63101
Phone: (314) 552 – 6307
Fax: (314) 552-7307